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(71) Applicant: BIOSOURCE TECHNOLOGIES, INC. [US/US]; 3333 Vaca Valley Parkway, Vacaville, CA 95688 (US).		
(72) Inventors: KUMAGAI, Monto, H.; 1330 Brown Drive, Davis, CA 95616 (US). DELLA-CIOPPA, Guy, R.; 814 Derry Circle, Vacaville, CA 95688 (US). DONSON, Jonathan; 717 Alvarado Avenue #233, Davis, CA 95616 (US). HARVEY, Damon, A.; 409 Eagle Lane, Vacaville, CA 95687 (US).		
(74) Agents: HALLUIN, Albert, P. et al.; Pennie & Edmonds, 1155 Avenue of the Americas, New York, NY 10036 (US).		<p><b>Published</b> With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</p> <p>(88) Date of publication of the international search report: 1 February 1996 (01.02.96)</p>

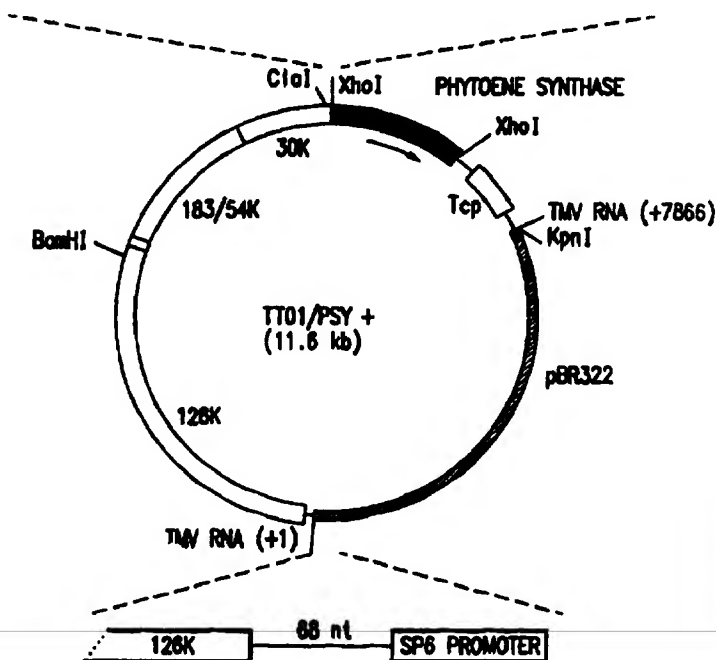
(54) Title: THE CYTOPLASMIC INHIBITION OF GENE EXPRESSION

## (57) Abstract

One aspect of the invention is to provide novel genetic constructions for the expression of inhibitory RNA in the cytoplasm of eukaryotic cells. The inhibitory RNA may be an anti-sense RNA or a co-suppressor RNA. The genetic constructions of the invention are capable of replicating in the cytoplasm of a eukaryotic cell and comprise a promoter region in functional combination with an encoding polynucleotide. The genetic constructions may be designed so as to replicate in the cytoplasm of plant cells, yeast cells, and mammalian cells. When the eukaryotic cell of interest is a plant cell, the genetic construction is preferably derived from a plant RNA virus. Plant RNA virus derived genetic constructions may employ a plant virus subgenomic promoter, including subgenomic promoters from tobamoviruses in functional combination with the RNA encoding region. Another aspect of the invention is to provide cells comprising the genetic constructions of the invention and organism comprising a plurality of such cells. Another aspect of the invention is to provide methods of reducing the expression of a gene of interest in eukaryotic cells, i.e., methods of producing eukaryotic cells exhibiting reduced levels of expression of a gene of interest. The methods of the invention comprise the step of transfecting a cell with a genetic construction of the invention in which the RNA encoding region is specific for the gene of interest. Another aspect of the invention is to provide plant cells that produce elevated levels of the carotenoid phytoene. The elevated levels of phytoene are achieved by inhibiting the expression at the enzyme phytoene desaturase using the vectors of the invention.

.tsp XhoI Start codon  
GTTTAAATAGGCTCGAGGTTTAAAT ATG TCT GTT GCC TTG TTA TCG GTT GTT TCT CCT TGT GAC  
Met Ser Val Ala Leu Leu Trp Val Val Ser Pro Cys Asp

TRANSIT PEPTIDE OF PHYTOENE SYNTHASE  
GTC TCA AAT GGC ACA AGT TTC ATG GAA TCA GTC CCG GAG CGA AAC CGT  
Val Ser Asn Gly Thr Ser Phe Met Glu Ser Val Arg Glu Gly Asn Arg



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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 95/06741

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/83 C12N15/11 C12N15/53 C12N15/52 C12N5/10  
A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VIRUS RESEARCH, vol. 32, no. 1, 1994 pages 57-67, POWERS, A.M., ET AL. 'INTRACELLULAR IMMUNIZATION OF MOSQUITO CELLS TO LACROSSE VIRUS USING A RECOMBINANT SINDBIS VIRUS VECTOR' see the whole document ---	1, 2, 9, 10, 17, 18, 31, 32
X	WO, A, 93 03161 (DONSON JON ; DAWSON WILLIAM O (US); GRANTHAM GEORGE L (US); TURPEN) 18 February 1993 see page 23, line 21 - line 25 see page 30, last paragraph - page 31 --- -/--	1-38

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+ 31-70) 340-3016

Authorized officer

Maddox, A

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A	THE PLANT JOURNAL, vol. 2, no. 3, 1992 pages 343-349, BRAMLEY, P., ET AL. 'BIOCHEMICAL CHARACTERIZATION OF TRANSGENIC TOMATO PLANTS IN WHICH CAROTENOID SYNTHESIS HAS BEEN INHIBITED THROUGH THE EXPRESSION OF ANTISENSE RNA TO PTOM5' see the whole document ---	
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International Application No

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